

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application. Inserted text is indicated with underlining and deleted text is indicated with ~~striketrough~~. Claim status is indicated as **currently amended**, **original** or **cancelled**. These claim amendments and the new claims do not introduce new matter.

Listing of the Claims:

1. – 39. (Cancelled)

40. (Currently Amended) A eukaryotic cell comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an orthogonal tRNA (O-tRNA) with at least one unnatural amino acid in the eukaryotic cell, which unnatural amino acid comprises an alkynyl moiety or an azido moiety, wherein:

- (a) the O-RS or a portion thereof is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 20-35, ~~a complementary polynucleotide sequence thereof, or a conservative variant thereof;~~
- (b) the O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 48-63, ~~or a conservative variant thereof; or,~~
- (c) the O-RS comprises an amino acid sequence that is at least 90% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS; aspartate at a position corresponding to Asn126 of E. coli TyrRS; asparagine at a position corresponding to Asp182 of E. coli TyrRS; alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS; ~~or,~~
- ~~(d) the O-RS aminoacylates the O-tRNA with the at least one unnatural amino acid at least 50% as efficiently as does an O-RS having an amino acid sequence as set forth in SEQ ID NO.: 45.~~

41. **(Currently Amended)** The cell of claim 40, further comprising an orthogonal tRNA (O-tRNA), wherein the O-tRNA recognizes a selector codon and is preferentially aminoacylated with the at least one unnatural amino acid by the O-RS, wherein the O-tRNA is produced in a cell by cellular processing of a nucleic acid corresponding to SEQ ID NO.:65, and the O-RS comprises a polypeptide sequence selected from the group consisting of: SEQ ID NO.: 48-63, ~~and a conservative variation thereof.~~

42. **(Currently Amended)** A polypeptide selected from the group consisting of:

- (a) a polypeptide that comprises an amino acid sequence as shown in any one of SEQ ID NO.: 48-63;
- (b) a polypeptide that comprises an amino acid sequence encoded by a polynucleotide sequence as shown in any one of SEQ ID NO.: 20-35;
- (c) a polypeptide that is specifically immunoreactive with an antibody specific for a polypeptide of (a), or (b);
- (d) a polypeptide that comprises an amino acid sequence that is at least 90% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS; aspartate at a position corresponding to Asn126 of E. coli TyrRS; asparagine at a position corresponding to Asp182 of E. coli TyrRS; alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS; and,
- (e) a polypeptide that comprises at least 20 contiguous amino acids of SEQ ID NO.: ~~36-48, or 86, 48-63~~ and two or more amino acid substitutions selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS, aspartate at a position corresponding to Asn126 of E. coli TyrRS, asparagine at a position corresponding to Asp182 of E. coli TyrRS, alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS, and methionine,

valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS; ~~and,~~

~~(f) an amino acid sequence comprising a conservative variation of (a), (b), (c), (d), or (e).~~

43. (Original) A composition comprising the polypeptide of claim 42 and an excipient.

44.- 46. (Cancelled)

47. (Currently Amended) A polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence as set forth in any one of SEQ ID NO.: 20-35;

(b) a polynucleotide that is complementary to or that encodes a polynucleotide sequence of (a);

(c) a polynucleotide encoding a polypeptide that comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 48-63, ~~or a conservative variation thereof;~~

(d) a polynucleotide that encodes a polypeptide of claim 42;

(e) a nucleic acid that hybridizes to a polynucleotide of (a), (b), (c), or (d) under highly stringent conditions over substantially the entire length of the nucleic acid;

(f), a polynucleotide that encodes a polypeptide that comprises an amino acid sequence that is at least 90% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more mutations selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS, aspartate at a position corresponding to Asn126 of E. coli TyrRS, asparagine at a position corresponding to Asp182 of E. coli TyrRS, alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS, and methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS; and,

(g) a polynucleotide that is at least 98% identical to a polynucleotide of (a), (b), (c), (d), (e), or (f); ~~and,~~

~~(h) a polynucleotide comprising a conservative variation of (a), (b), (c), (d), (e), (f), or (g).~~

48. (Original) A vector comprising a polynucleotide of claim 47.

49. (Original) The vector of claim 48, wherein the vector comprises a plasmid, a cosmid, a phage, or a virus.

50. (Original) The vector of claim 48, wherein the vector is an expression vector.

51. (Original) A cell comprising the vector of claim 48.

52. – 61. (Cancelled)